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19 ABSTRACT (Continue on reverse if necessary and identify by block number) Our objectives have been to understand the role that methanogenic bacteria play in metal corrosion. We have demonstrated their ability to use Fe <sup>+</sup> , Al <sup>+</sup> , Zn <sup>+</sup> , Co <sup>+</sup> and Ni <sup>+</sup> (to some degree) as electron donors in methanogenesis, which suggests their possible role in biocorrosion; we have further studied the environmental conditions affecting the rates of metal oxidation. We have also studied key methanogenic enzymes involved in electron consumption (formate dehydrogenase, hydrogenase and methylene THMP dehydrogenase). The effects of alkyl tins on methanogens was examined, due to the use of one such compound, tributyl tin, in anticorrosion paints as a biocide. We find that many organotins are more toxic to methanogens than tributyl tin and that the relationship of hydrophobicity of the organotin to toxicity is quite different than observed by others in studies of aerobic organisms.														
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ANNUAL REPORT ON CONTRACT N00014-88-K-0195

PRINCIPAL INVESTIGATOR: Lacy Daniels

CONTRACTOR: The University of Iowa

CONTRACT TITLE: Archaebacterial Involvement in Microbial Metal Corrosion: Metabolism of S and Fe

START DATE: 1 February 1988

RESEARCH OBJECTIVES: To understand the metabolism of  $Fe^0$  and  $S^0$  by archaeabacteria and how it relates to biocorrosion; understand related electron transfer reactions in methanogens; understand inhibition of methanogens by organotins.

PROGRESS Year 2:

Use of metals other than Fe. We have concluded a series of experiments demonstrating that  $Zn^0$ ,  $Al^0$ ,  $Co^0$  and  $Ni^0$  can serve as electron donors for methanogenesis. This work has been published in Antonie von Leeuwenhoek (see Publications list below).

Inhibition of Methanogens by alkyl tins. Tributyl tin is used as a microbial inhibitor in antifoulant and anticorrosion paints commonly used on ships and piers. We have investigated the effects of tributyl tin and related organotins on methanogens and other anaerobes thought to play a role in biocorrosion.

Methyl and butyltin compounds were inhibitory to all anaerobes examined, but there were great variations, depending on the specific alkyltin and bacterium. The methanogens were inhibited more strongly by methyl than by butyl derivatives: more than 50% inhibition occurred with 0.025-0.5 mM of the methyltins, whereas 0.16-1.8 mM butyltins were needed for the same level of inhibition: tri-butyltin was the least toxic. *Methanosarcina barkeri* was, in general, more resistant than the *Methanococcus* sp. and *Methanobacterium bryantii*. The *Desulfovibrio* were more strongly inhibited by mono-methyltin than by di- and tri-methyl derivatives; butyltins were, in general, not so toxic. Mono-methyltin at 0.15 mM almost completely inhibited three of the sulfate reducers, but *Desulfovibrio thermophilus* required 0.7 mM for this level of inhibition. Tri-butyltin at 1.8 mM did not cause major inhibition, whereas mono- and di-butyltins were more inhibitory. *Acetobacterium woodii* was most affected by mono- and dimethyltins, and least by tri-methyltin and mono-butyltin. In contrast, *Wolinella succinogenes* was most affected by tri-methyltin. This study suggests several major groups of anaerobes thought to be involved in metal biocorrosion vary greatly in their response to alkyltins; most interesting is the relative insensitivity by methanogens and sulfate reducers to tri-butyltin, which is a major component in commercial antifouling paints. Our results differ considerably from those reported for aerobic microorganisms, which were found to be most affected by tributyltin.

This work has been accepted for publication in Current Microbiology.

Codes

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A-1

A more complete study using a wider range of sizes of organo groups is currently being completed to further examine the relationship of the hydrophobicity of organotins to their toxicity to methanogens.

Regulation of Formate Dehydrogenase and Hydrogenase. This work has recently been published in *J. Bacteriology* (see Publications list below).

Effect of  $\text{PO}_4$  concentration on utilization of cathodic hydrogen. With increase in phosphate concentration (0.1 to 20 mM), there was increased chemical production of cathodic hydrogen from mild steel in methanogen medium free of cells. At the end of 15 days of incubation at 37°C, about 62  $\mu\text{moles}$  of hydrogen/tube was produced with a phosphate concentration of 20 mM, while only about 14  $\mu\text{moles}$  were produced with 0.1 mM phosphate. At 64°C there was at least two-fold higher production of hydrogen. *Methanosarcina barkeri*, *Methanospirillum hungatei*, *Methanobacterium thermoautotrophicum* (Marburg) and *Methanococcus thermolithotrophicus* were able to utilize cathodic hydrogen at all concentrations of phosphate. Thus, there was significantly higher production of methane with increasing concentrations of phosphate; this implies a higher metal corrosion rate at higher phosphate levels; phosphate (up to 20 mM) did not have any significant effect on normal growth (under  $\text{H}_2\text{-CO}_2$  of *Methanosarcina barkeri*, *Ms. hungatei*, *Mb. thermoautotrophicum* (Marburg) and *Mc. thermolithotrophicus*). Weight loss studies of mild steel coupons with *Ms. barkeri* also gave higher mdd (mg/day/dm<sup>2</sup>) values for phosphate concentration of 20 mM (4.7 mdd at the end of 30 days) as opposed to that of 0.1 mM phosphate (1.2 mdd at the end of 30 days).

Effect of NaCl on utilization of cathodic hydrogen. Increasing concentrations of NaCl (0.9 to 59 mM) also induced significantly higher production of cathodic hydrogen from mild steel. At the end of 15 days at 37°C, 27  $\mu\text{moles}$  of cathodic hydrogen/tube was produced with NaCl concentration of 59 mM, while it was only about 11  $\mu\text{moles}$  with 0.9 mM NaCl. At 64°C, there was 2-3 fold higher production of cathodic hydrogen. *Methanosarcina barkeri* and *Methanobacterium thermoautotrophicum* (Marburg) were able to utilize cathodic hydrogen at all concentrations of NaCl. NaCl (up to 104 mM) did not have any effect on normal growth of *Methanosarcina barkeri* and *Methanobacterium thermoautotrophicum*.

#### WORK PLAN:

We have had a change of personnel and a partial shift of thrust in one part of the project. Mr. N. Belay has now shifted to an entirely different project to begin his Ph.D. work. Mr. R. Sparling has finished his Ph.D. and is now a postdoctoral fellow at Gottingen, West Germany. Dr. Rajogopal, who has been on the project for 3-4 years, has gotten married and moved to be with his wife at the University of Minnesota. He has been replaced with Dr. Boopathy, who comes from the University of Missouri. Through Dr. Rajogopal's visit last year to work with the French-Georgia group (Le Galle, Fauque et al.), we have become aware that they are concentrating part of their effort on the study of Sulfur-reducing enzymes; their lab is very well equipped for this, and we will not try to duplicate their efforts, but will concentrate on methanogen corrosion activities, using our pure cultures, and isolates we plan to obtain from coastal corrosion environments. We have collaborated with the French group in writing a review on microbial sulfur metabolism (see Publication list below). We will also study the regulation of methanogen electron consumption and

transfer activities (e.g., see above on formate dehydrogenase and hydrogenase) since they are essential components of the metal oxidation-corrosion activities. These enzyme regulation studies and pure culture biocorrosion studies described in our proposal are more consistent with our current skills, and allow a good equitable distribution of research activity between ourselves and the French-Georgia groups. In particular, during the next 6 months we will study methanol and acetate use by *Methanosarcina*, correlated with methylene THMP dehydrogenase activity. We will also conclude our studies on the effects of phosphate, salt and pH on methanogenesis using  $Fe^0$  as the electron source. We will also conclude our whole cell nutritional studies on the  $NO_3^-$  metabolism by methanogens. It is our intent to further pursue in a renewal application the enzymology of the nitrate reductase, due to its unusual metal requirement.

INVENTIONS: None

PUBLICATIONS:

Belay, N. and L. Daniels. 1990. Elemental metals as electron sources for biological methane formation from  $CO_2$ . *Anton. van Leeuwenhoek.* 57:1-7.

Belay, N., B. S. Rajagopal, and L. Daniels. 1990. Effects of alkyltin compounds on hydrogen-oxidizing anaerobic bacteria. *Curr. Microbiol.* 20: -.

LeFaou, A., B. S. Rajagopal, L. Daniels and G. Fauque. 1990. Thiosulfate, polythiomates and elemental sulfur assimilation and reduction in the bacterial world. *FEMS Microbiology Rev.* (In press).

Belay, N., K.-Y. Jung, B. S. Rajagopal, J. D. Kremer and L. Daniels. 1990. Nitrate as a sole nitrogen source for *Methanococcus thermolithotrophicus* and its effect on growth of several methanogenic bacteria. *Curr. Microbiol.* (In press).

Sparling, R. and L. Daniels. 1990. Regulation of formate dehydrogenase activity in *Methanococcus thermolithotrophicus*. *J. Bacteriol.* 172: -.

TRAINING ACTIVITIES:

Dr. Rajagopal has finished his last year in our lab, and is now at the University of Minnesota. Mr. N. Belay has become a graduate student in this lab on a non-ONR project, but continues to interact scientifically with our activities. Mr. Biswarup Mukhopadhyay is a final year graduate student continuing on the regulation of electron consuming methanogenic reaction studies. Dr. R. Boopathy is now the major researcher on both alkyl tin aspects and  $Fe^0$  driven methanogenesis.

Women or Minorities - 0

Non-Citizens - 3 citizens of India  
- 1 U.S. permanent resident (Ethiopian decent)

AWARDS/FELLOWSHIPS: None